

SEQUENCE LISTING



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<120> Modifications of Apoptin

<130> 2906-4996.1

<140> 10/083,849

<141> 2001-10-19

<150> US 60/242,397

<151> 2000-10-20

<160> 20

<170> PatentIn version 3.1

<210> 1

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MISC_FEATURE

<222> (1)..(121)

<223> Apoptin (a small protein derived from chicken anemia virus) encoded by pCMV-Vp3 and by GFP-Apoptin constructs

<400> 1

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
 1 5 10 15
 Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
 20 25 30
 Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
 35 40 45
 Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
 50 55 60
 Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
 65 70 75 80
 Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
 85 90 95
 Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro
 100 105 110
 Arg Thr Ala Arg Arg Arg Ile Arg Leu
 115 120

<210> 2

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> Apoptin protein encoded by pIRESneo alanine mutants

<220>

<221> MISC_FEATURE

<223> Differs from Apoptin protein encoded by pCMV-vp3 and by GFP-Apoptin constructs by replacement of the arginine residue at position 116 with a lysine residue

<400> 2

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Lys Arg Arg Ile Arg Leu
115 120

<210> 3

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-86 of 5-alanine linker-scanning mutant series of Ap
optin

<400> 3

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Ala Ala Ala Ala Ala Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 4

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-91 of 5-alanine linker-scanning mutant series of Ap
optin

<400> 4

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
4

65		70		75		80									
Pro	Lys	Pro	Pro	Ser	Lys	Lys	Arg	Ser	Cys	Ala	Ala	Ala	Ala	Ala	Arg
				85					90					95	
Val	Ser	Glu	Leu	Lys	Glu	Ser	Leu	Ile	Thr	Thr	Thr	Pro	Ser	Arg	Pro
			100					105					110		
Arg	Thr	Ala	Arg	Arg	Arg	Ile	Arg	Leu							
		115					120								

<210> 5

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-96 of 5-alanine linker-scanning mutant series of Ap
optin

<400> 5

Met	Asn	Ala	Leu	Gln	Glu	Asp	Thr	Pro	Pro	Gly	Pro	Ser	Thr	Val	Phe
1				5					10					15	
Arg	Pro	Pro	Thr	Ser	Ser	Arg	Pro	Leu	Glu	Thr	Pro	His	Cys	Arg	Glu
			20					25					30		
Ile	Arg	Ile	Gly	Ile	Ala	Gly	Ile	Thr	Ile	Thr	Leu	Ser	Leu	Cys	Gly
		35					40					45			
Cys	Ala	Asn	Ala	Arg	Ala	Pro	Thr	Leu	Arg	Ser	Ala	Thr	Ala	Asp	Asn
	50					55					60				
Ser	Glu	Ser	Thr	Gly	Phe	Lys	Asn	Val	Pro	Asp	Leu	Arg	Thr	Asp	Gln
65					70					75					80
Pro	Lys	Pro	Pro	Ser	Lys	Lys	Arg	Ser	Cys	Asp	Pro	Ser	Glu	Tyr	Ala
				85					90					95	
Ala	Ala	Ala	Ala	Lys	Glu	Ser	Leu	Ile	Thr	Thr	Thr	Pro	Ser	Arg	Pro
			100					105					110		

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 6

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-101 of 5-alanine linker-scanning mutant series of A
poptin

<400> 6

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Ala Ala Ala Ala Ala Thr Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 7

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-106 of 5-alanine linker-scanning mutant series of A
poptin

<400> 7

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Ala Ala Ala Ala Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 8

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-111 of 5-alanine linker-scanning mutant series of A
poptin

<400> 8

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Ala Ala
100 105 110

Ala Ala Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 9

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-116 of 5-alanine linker-scanning mutant series of A
poptin

<400> 9

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Ala Ala Ala Ala Ala Leu
115 120

<210> 10

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T106A of Apoptin

<400> 10

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 11

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T107A of Apoptin

<400> 11

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Ala Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 12

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T108A of Apoptin

<400> 12

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Ala Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
 115 120

<210> 13

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant P109A of Apoptin

<400> 13

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Ala Ser Arg Pro
 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
 115 120

<210> 14

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T106E of Apoptin

<400> 14

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Glu Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 15

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T107E of Apoptin

<400> 15

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Glu Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 16

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T108E of Apoptin

<400> 16

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Glu Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 17

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutation T106A107A of Apoptin

<400> 17

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Ala Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 18

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutant T107A108A of Apoptin

<400> 18

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Ala Ala Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 19

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutant T106A108A of Apoptin

<400> 19

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Thr Ala Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 20

<211> 8

<212> PRT

<213> Chicken anemia virus

<220>

<221> MISC_FEATURE

<223> amino acid sequence encoding the SV40-Large T nuclear localization signal

<400> 20

Pro Pro Lys Lys Lys Arg Lys Val
1 5